

MAY 31 1988

AD-A195 902

Brief Reports

EVIDENCE OF HUMAN INFECTION WITH A RAT-ASSOCIATED
HANTAVIRUS IN BALTIMORE, MARYLANDJAMES E. CHILDS,¹ GREGORY E. GLASS,¹ GEORGE W. KORCH,¹ RAY R. ARTHUR,¹
KEERTI V. SHAH,¹ DAVID GLASSER,² CYNTHIA ROSSI,³ AND JAMES W. LEDUC¹

Viruses of the proposed genus *Hantavirus* (in the family Bunyaviridae) are etiologic agents of hemorrhagic fever with renal syndrome in Asia and Europe (1, 2). Four antigenically distinct hantaviruses have been isolated from different rodent reservoirs, and three are associated with hemorrhagic fever with renal syndrome in humans (3). The primary virus-rodent associations and corresponding human diseases are Hantaan virus-*Apodemus agrarius* with Korean hemorrhagic fever and severe-type epidemic hemorrhagic fever in eastern Asia (1, 4); Puumala virus-*Clethrionomys glareolus* with nephropathia epidemica in eastern Europe, western Soviet Union, and Scandinavia (2); and Seoul virus (and other isolates) and species of *Rattus* with mild-type epidemic hemorrhagic fever in China and in laboratory outbreaks of hemorrhagic fever with renal syndrome (4-6). Prospect Hill virus, isolated from *Microtus pennsylvanicus* in the United States, is known to

infect humans, but is not associated with a disease (7).

Recently, hantaviral infections in wild *Rattus norvegicus* of the United States were documented and shown by virologic and serologic techniques to be caused by a virus antigenically related to Seoul virus, isolated in 1980 from a Norway rat in Korea (5, 8-10). The prevalence of this infection in the rats of Baltimore, Maryland, is high, geographically widespread, and enzootic (10). We now report serologic evidence of human infections specifically due to a rat-associated *Hantavirus* in residents of Baltimore. To our knowledge, this is the first report to definitively link to a rat source the occurrence of hantaviral antibodies in humans who are lifelong residents of the United States.

MATERIALS AND METHODS

Subjects

A total of 1,856 serum samples were obtained from 1,788 persons (two samples were obtained from 68 persons) from two sources in Baltimore. Sera were collected on a weekly schedule from January 1985 through July 1986 from persons visiting a sexually transmitted disease clinic operated by the Baltimore City Department of Health. A total of 1,478 samples (71.7 per cent male) were gathered from 1,459 persons with a mean age of 26.9 ± 7.7 (standard deviation) years and ranging in age from 13-71. Age, sex, and household address specific to block number were obtained for each person, but no clinical records or personal histories were available for this population. The area serviced by this

Received for publication June 30, 1987.

¹ Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, MD 21205. (Reprint requests to Dr. James E. Childs.)

² Bureau of Disease Control, Baltimore City Department of Health, 111 N. Calvert Street, Baltimore, MD.

³ Department of Epidemiology, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

This research was supported by contracts DAMD17-84-C-4015 and DAMD17-87-C-7101 from the United States Army Medical Research and Development Command.

The authors thank Dr. James Meegan and Carol Robinson for help with the enzyme-linked immunosorbent assay. Drs. Joel Dalrymple and Tom Ksiazek made useful comments on earlier drafts of this paper.

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

clinic included many neighborhoods known to harbor infected rats (10). A total of 378 sera from 329 patients (36.4 per cent male) at The Johns Hopkins Hospital with proteinuria levels equal to or greater than 250 mg per 24 hours were collected from March through July 1986. Proteinuria is a common laboratory finding in patients with mild and severe forms of hemorrhagic fever with renal syndrome and was chosen as a marker for identifying potentially infected persons (11). Subjects from The Johns Hopkins Hospital had a mean age of 42.5 ± 21.1 years and ranged in age from 2-95. Clinical records and personal histories were available for hospital subjects and were examined for evidence of disease compatible with hemorrhagic fever with renal syndrome or indication of foreign travel to locations endemic for the disease.

Serologic tests

An indirect immunofluorescent assay using Vero E-6 cells (ATCC no. CRL 1586) infected with prototype Hantaan virus, strain 76-118 (prepared by Dr. George French, Salk Institute, Swiftwater, PA) as an antigen source was utilized to screen sera at dilutions of 1:8 and 1:32 for virus-specific antibody (8, 10). Sera positive at 1:32 were titrated to endpoint. Previous experience proved Hantaan virus to be a sensitive test antigen for detecting antibody in naturally infected rats and humans infected with various hantaviruses (8, 10). Sera positive by immunofluorescent assay and 35 negative sera were further tested by an enzyme-linked immunosorbent assay and by constant virus plaque-reduction neutralization tests. In the enzyme-linked immunosorbent assay, Hantaan viral antigen was obtained from supernatant fluids of infected Vero E-6 cell cultures and bound to wells of microtiter plates (Immulon II, Dynatech, Alexandria, VA) coated with Hantaan-specific mouse monoclonal antibodies (hybridomas prepared by Dr. J. B. McCormick, Centers for Disease Control, Atlanta, GA). Supernatant fluid from uninfected cell cultures was used as a control

antigen. Serum samples were screened at a dilution of 1:100, and goat anti-human immunoglobulin G conjugated with alkaline phosphatase (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) was used as the detector antibody. Optical densities were determined spectrophotometrically after adding *p*-nitrophenyl phosphate. Samples were considered positive for Hantaan-specific antibody if the differences in optical density between Hantaan and control antigen wells were at least three standard deviations above the mean difference in optical density obtained from three negative control sera. All positive sera were titrated to endpoint. The neutralization tests utilized Baltimore rat virus (10), Hantaan virus (1), and Prospect Hill virus (strain MP-40) (12). Differences greater than fourfold in 80 per cent neutralization titers to the three hantaviruses were used to interpret the specificity of the antibody response and to indicate the probable virus-rodent source of infection.

RESULTS

Sera from four subjects (three from The Johns Hopkins Hospital and one from the sexually transmitted disease clinic) were positive for *Hantavirus*-specific antibodies by immunofluorescent assay, enzyme-linked immunosorbent assay, and neutralization tests (table 1). Highest titers of neutralizing antibody were detected to Baltimore rat virus, with titers ranging from eight- to greater than 16-fold higher compared with prototype Hantaan virus. All sera lacked neutralizing antibody to Prospect Hill virus. No sera negative by immunofluorescent assay reacted in any additional assay. The serologic pattern seen with these human samples was similar to that seen among naturally infected Baltimore rats and distinct from that seen in patients convalescing from Korean hemorrhagic fever (table 1). These results suggest that a rat-associated *Hantavirus* was responsible for the infections.

Two men, aged 27 and 40 years and two women aged 49 and 87 years were seropos-

TABLE 1

Antibodies to Hantaan virus, Baltimore rat virus, and Prospect Hill virus in four persons from Baltimore, Maryland, as measured by immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA), and plaque reduction neutralization (PRN) test, January 1985 to July 1986

Human study group	Patient	Antibody titer*				
		IFA (Hantaan virus)	ELISA (Hantaan virus)	PRN		
				Baltimore rat virus	Hantaan virus	Prospect Hill virus
The Johns Hopkins Hospital	1	256	3,400	1,024	64	<16
	2	128	16,000	1,024	128	<16
	3	512	1,800	160	<16	<16
Sexually transmitted disease clinic	4	2,048	32,000	>2,048	256	<16
Wild rat infected with Baltimore rat virus		1,096	ND†	>2,048	128	<16
Convalescent serum from patient with Korean hemorrhagic fever		1,024	8,000	<16	128	<16

* Titers are expressed as reciprocal values, and PRN titers are those resulting in $\geq 80\%$ reduction in the number of viral plaques.

† ND, not done.

itive. Medical records from two of the three Johns Hopkins Hospital patients (aged 40 and 49 years) indicated lifelong residence in Baltimore and an absence of foreign travel. The third hospital subject had lived her entire life in Baltimore, but records regarding foreign travel could not be obtained. The positive serum collected at the sexually transmitted disease clinic was from a 27-year-old male with an unknown history of travel. The occurrence of a clinical illness compatible with hemorrhagic fever with renal syndrome could not be established for any person.

An additional 52 sera were positive by immunofluorescent assay, of which five were also positive by enzyme-linked immunosorbent assay at titers $\geq 1:200$, but neutralization results were uniformly negative for these sera.

DISCUSSION

Although *Hantavirus*-specific antibody has been reported in humans in North America (7, 9, 13-15), only one study has implicated a specific virus-rodent source for the infections (7). This association was for Prospect Hill virus and the presence of antibody in a group of professional mammalogists, many of whom had close contact with *M. pennsylvanicus* during the course

of field investigations. Our data indicate that human infections with a rat-associated *Hantavirus* are acquired within Baltimore. More detailed epidemiologic studies are under way to document the types of exposure resulting in infection and potential occurrence of clinical disease.

Hantaviruses of rats cause human disease in Asia, with symptoms ranging from mild influenza-like illness to severe hemorrhagic fever with renal syndrome, occasionally leading to death (4). The protean nature of this disease may make diagnosis difficult outside recognized endemic areas. The high prevalence of hantaviral infections in urban rats in Baltimore and other cities in the United States (8-10), the existence of human disease resulting from contact with rat-associated hantaviruses in other parts of the world (4, 5), and our finding that humans have antibodies from infections acquired within the United States all indicate that epidemiologic monitoring of the domestic situation is warranted.

SUMMARY

Human sera obtained from two sources in Baltimore were tested for antibodies against a *Hantavirus* by a variety of techniques. Four persons out of 1,788 had han-

INSPECTED 2

☒ ☐ ☐

Codes

nd/or

tal

A-1 21

taviral antibody as demonstrated by immunofluorescent assay, enzyme-linked immunosorbent assay, and neutralizing assay. Neutralizing antibody titrations against three test viruses indicated that infections were caused by a rat-associated virus. Medical histories of two subjects indicated life-long residence in Baltimore and an absence of foreign travel, implicating a local source of infection.

REFERENCES

1. Lee HW, Lee PW, Johnson KM. Isolation of the etiologic agent of Korean hemorrhagic fever. *J Infect Dis* 1978;137:298-308.
2. Brummer-Korvenkontio M, Vaheri A, Hovi T, et al. Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. *J Infect Dis* 1980;141:131-4.
3. Schmaljohn CS, Hasty SE, Dalrymple JM, et al. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science* 1985;227:1041-4.
4. Song G, Hang C-S, Qui X-Z, et al. Etiologic studies of epidemic hemorrhagic fever (hemorrhagic fever with renal syndrome). *J Infect Dis* 1983;147:654-9.
5. Lee HW, Baek LJ, Johnson KM. Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. *J Infect Dis* 1982;146:638-44.
6. Lee HW, Johnson KM. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. *J Infect Dis* 1982;146:645-51.
7. Yanagihara R, Gajdusek DC, Gibbs CJ Jr, Prospect Hill virus: serologic evidence for infection in mammalogists. *N Engl J Med* 1984;310:1325-6.
8. LeDuc JW, Smith GA, Johnson KM. Hantaan-like viruses from domestic rats captured in the United States. *Am J Trop Med Hyg* 1984;33:992-8.
9. Tsai TF, Bauer SP, Sasso DR, et al. Serological and virological evidence of a Hantaan virus-related enzootic in the United States. *J Infect Dis* 1985;152:126-36.
10. Childs JE, Korch GW, Glass GE, et al. Epizootiology of *Hantavirus* infections in Baltimore: isolation of a virus from Norway rats, and characteristics of infected rat populations. *Am J Epidemiol* 1987;126:55-68.
11. McKee KT Jr, MacDonald C, LeDuc JW, et al. Hemorrhagic fever with renal syndrome—a clinical perspective. *Milit Med* 1985;150:640-7.
12. Lee PW, Amyx HL, Yanagihara R, et al. Partial characterization of Prospect Hill virus isolated from meadow voles in the United States. *J Infect Dis* 1985;152:826-9.
13. Gibbs CJ Jr, Takenaka A, Franko M, et al. Seroepidemiology of Hantaan virus. *Lancet* 1982;2:1406-7.
14. Lee HW, Seong IW, Baek LJ, et al. Positive serological evidence that Hantaan virus, the etiologic agent of hemorrhagic fever with renal syndrome, is endemic in Canada. *Can J Microbiol* 1984;30:1137-40.
15. Yanagihara R, Chin C-T, Weiss MB, et al. Serological evidence of Hantaan virus infection in the United States. *Am J Trop Med Hyg* 1985;34:396-400.